

CALIFORNIA INSTITUTE OF TECHNOLOGY  
PASADENA

GATES AND CRELLIN LABORATORIES OF CHEMISTRY

October 28, 1958

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Dear Lu:

Lu, you are the victim of my  
first encounter with a dictaphone. Forbearance!

Your Jean is in Mexico resting after a rather hectic month in Pasadena with the many persons who are visiting currently. Therefore I will try to tell you some of the technical details of our experiment with lambda in cesium chloride density gradients.

First of all, you should obtain your cesium chloride from the American Potash Company, 3030 W. Sixth Street, Los Angeles 54. You should direct your correspondence to Mr. Frank Radovitch. The cesium chloride supplied by American Potash is not completely pure but contains about 3% rubidium. This contamination with rubidium, however, should not trouble your work with viruses. Its only effect, so far as we know, is to very slightly change the actual density gradient from what one ~~might~~ <sup>would</sup> expect if the salt were pure cesium chloride.<sup>†</sup>

This change is only a percent or so and can be neglected. The cesium chloride from American Potash costs about 25 dollars a pound. You should treat it very carefully overnight with a small amount of acid-washed decolorizing charcoal. This should take the optical density of the saturated cesium chloride solution down below 0.1. You will find data for the composition and density of cesium chloride solutions at various temperatures in the International Critical Tables. The density of lambda is 1.51 grams per cubic centimeter. We usually work by diluting a stronger cesium chloride solution with a standard amount of lambda sample or water to give a final density of 1.51. Also present in the cesium chloride solution which we use is 0.01 molar tris buffer and the solution is brought to pH 7 as determined by a glass electrode pH meter before use. We have found in this solution the titre of lambda remains constant at 20° even for many days of exposure. If you want to find the density of a new virus in cesium chloride, I would recommend that you place a great deal of it, let us say enough to give it optical density of 1, in the analytical cell with cesium chloride solution of density, perhaps 1.5, and watch to see which way the bulk of the material moves. Then you should adjust the density of the cesium chloride in the second experiment in the direction indicated by the first. Usually in a few tries this will enable you to determine the density quite accurately. In the case of lambda we centrifuge at 27,690 revolutions a minute. At this speed the time required for essential equilibrium is approximately 12 hours. I would recommend that in the

† For CsCl solutions centrifuged to equilibrium at 25°C, the value of  $\frac{\partial \rho}{\partial r}$  is  $8.0 \times 10^{-10} \omega^2 r$  gm cm<sup>-4</sup>.  $\omega$  is in radians sec<sup>-1</sup> and  $r$  in cm. This value holds at  $\rho = 1.70$ . For  $1.70 \gg \rho \gg 1.4$  it will be good to within 10% even at 20°C.

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analytical run you use plastic centerpieces as supplied by Spinco. In the case of the swinging bucket machine which we use for experiments from which we want to recover the lambda you must spin for a somewhat longer time at the same speed of 27, or 28,000 rpm. You should spin at least 16 hours to obtain equilibrium with respect to the cesium chloride and then you should continue spinning if ~~your virus~~ <sup>P-1</sup> takes longer than the length of time I mentioned for lambda when run in the analytical machine. Specifically, if ~~your virus~~ <sup>P-1</sup> takes longer than say, 12-14 hours to come to apparent equilibrium in the analytical machine then in the swinging bucket use a length of time increased by a factor of 2 over the time you find in the analytical cell. After a swinging bucket run the tubes which should be filled to ~~no more than~~ <sup>just</sup> 3 milliliters can be removed to a lab bench where a small hole is punctured in their bottoms and the falling drops are collected in separate test tubes. I would strongly advise you to practice this exercise several times before actually performing an experiment with virus. It is very easy to let the drops fall down the outside of the test tube. I can't think of any more details which would be of use to you off hand but if there is anything please let me know and I will write to you immediately.

Give my best regards to all the people at M. I. T.

Sincerely yours,

Matt

Matt Meselson

MM:rh